Western Blot

1. **Transfer Step**
    a. Transfer contents of SDS-Page gel to nitrocellulose membrane using western blot transfer apperatus
    b. Transfer Buffer (filter sterilize using 0.22 μm filter:
        - 20 mM Tris pH 8.0
        - 150 mM glycine
        - 30% v/v methanol
    c. Transfer for 1 hour at 250 Amps keeping the buffer cold with ice pack
2. **Blocking Step**
    a. Remove membrane from transfer apparatus
    b. Incubate the membrane in 25 mL blocking buffer, on rotating platform for 1 hour in the dark
    c. Blocking Buffer:
        - 25 mM Tris pH 8.0
        - 125 mM NaCl
        - 0.5% v/v Tween 20
        - 5% w/v skim milk powder
3. **Wash Step**
    a. Wash 2 times for 5 minutes in 25mM wash buffer, on rotating platform, in the dark
    b. Wash buffer:
        - 25 mM Tris pH 8.0
        - 125 mM NaCl
        - 0.5% v/v Tween 20
4. **Primary Antibody Incubation**
    a. Make a 1/30,000 dilution of the primary antibody (anti-RsaA) in 25 mL of blocking buffer
    b. Incubate the membrane the the mix, on rotating platform for 1 hour in the dark
5. **Wash Step**
    a. Wash 2 times for 5 minutes in 25mM wash buffer, on rotating platform, in the dark
6. **Secondary Antibody Incubation**
7. Make a 1/50,000 dilution of the secondary antibody (Goat-anti-rabbit w/ fluorophore) in 25 mL of blocking buffer
8. Incubate the membrane the the mix, on rotating platform for 1 hour in the dark
9. **Wash Step**
    a. Wash 2 times for 5 minutes in 25mM wash buffer, on rotating platform, in the dark
10. **Imaging**
    a. Use licor imager
    b. Excite the fluorophore at 700 nm wavelength